

Setting the stage: an anatomist's view of the immune system

Enrico Crivellato¹, Angelo Vacca² and Domenico Ribatti³

¹Department of Medical and Morphological Researches, Anatomy Section, University of Udine Medical School, P.le Kolbe n. 3, Udine, Italy

²Department of Biomedical Sciences and Human Oncology, University of Bari Medical School, P.le G. Cesare n. 11, Policlinico, Bari, Italy

³Department of Human Anatomy and Histology, University of Bari Medical School, P.le G. Cesare n. 11, Policlinico, Bari, Italy

Anatomical strategies that help cells to interact greatly increase the efficiency of the adaptive immune system. *In vivo*, antigens are presented in a complex environment, wherein their movements and those of antigen-presenting cells, T cells and B cells are subject to anatomical constraints. Specialized subcompartments appear to facilitate cell-to-cell contact and recognition and provide the most favorable milieu for signaling and induction mechanisms. How does the overall organization of a lymphoid organ facilitate the initiation and regulation of adaptive immune responses? This Review offers some answers to this basic question and focuses on the latest advances in our understanding of the functional anatomy of the lymph nodes, spleen and thymus.

The immunological scene is similar to a stage where a comedy or sometimes a tragedy is being performed. There are actors, both leading players and extras, a setting that often changes from one act to the next and obviously a plot. Like all biologists, anatomists are highly interested in the story but have difficulty in deciphering the strange and elusive language in which the lines of actors are spoken. They look for clues in the appearance of the players, their size, looks, gestures, and the shape and color of their costumes. In this search, they assign the greatest significance to the scenery, the wings, the setting, the seemingly unimportant furniture and all the devices whereby the actors meet and interact. This Review examines three immunological settings – the architecture and functional anatomy of the lymph nodes, spleen and thymus.

The functional anatomy of lymph nodes

Lymph nodes have the typical structure of peripheral lymphoid organs, with defined T- and B-cell-dependent compartments (Figure 1). They are encapsulated organs, presenting distinct cortical and medullary regions.

Organization of lymph node sinuses

The afferent lymph slowly and unceasingly flows through the subcapsular, intermediate and medullary sinuses and exits through the hilus as the efferent lymph. Sinuses are lined by sinus endothelial cells (SECs). These are flattened

cells that do not form a continuous layer, especially in the wall of the medullary sinus, but contain intercellular gaps or pores. Gaps have also been demonstrated in the floor of the subcapsular sinus. However, soluble lymph-borne material has restricted access to the cortex [1]. Experiments using fluorescent tracers have shown that molecules the diameter of which exceeds 7.2–10.7 nm are excluded from the cortex, whereas smaller molecules enter the cortical conduit system (see later).

The architecture of stromal cells

As typical lymphoid organs, lymph nodes have two components – the stroma with its fixed cells and the parenchyma with its migrating cell populations. Fibroblastic reticular cells (FRCs) constitute the main stromal population in the mammalian lymph node and form its

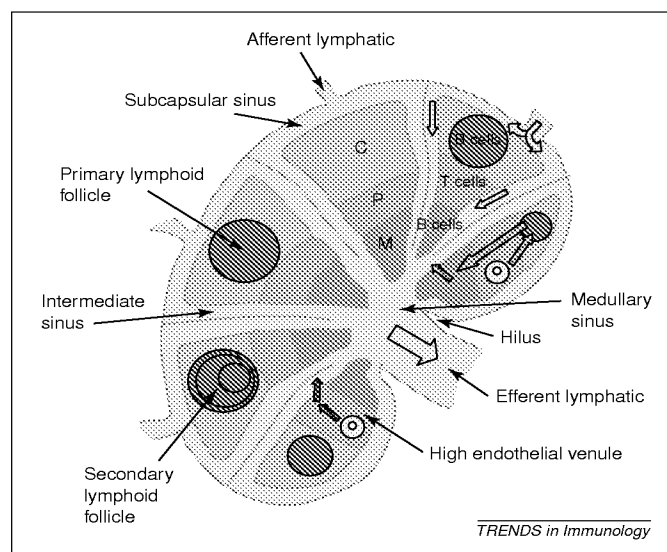


Figure 1. The general structure of a lymph node. C, P and M indicate the cortical, paracortical and medullary regions, respectively. The cortex is largely occupied by primary or secondary lymphoid follicles; together with medullary cords, they represent the B-cell compartment. By contrast, the interposed paracortical region is a T-cell-rich domain. The lymph enters the lymph node through multiple afferent lymphatics and exits the organ through a single draining vessel, the efferent lymphatic. Yellow arrows indicate the direction of the lymph flow, which runs from the subcapsular to the medullary sinus through intermediate sinuses. Green and blue arrows indicate migration of B and T cells from high endothelial venules to specific 'homing' compartments.

internal framework [2] (Figure 2). Their morphology, orientation and connections are determined by their location, mainly fusiform in the cortex and stellate in the medulla, where they form an extensive three-dimensional network [3,4] (Figure 3). FRCs have long been considered as merely supporting cells but it is becoming clear that they have additional functions. They express surface molecules and produce 'homing' chemokines for T cells, B cells and dendritic cells (DCs) [5–7]. They are also thought to provide anatomical arrangements that influence the traffic patterns of lymphocytes. Their spatial organization, in fact, facilitates lymphocyte 'crawling' along preformed 'corridors'. Moreover, they typically wrap reticular fibers (mainly made up of type III and IV collagen, elastin and laminin) to form a set of interconnected channels, called FRC conduit system [4,8], for the efficient and rapid transfer of soluble molecules, such as antigens, chemokines and immune complexes, from the subcapsular sinus to the deeper cortex, the high endothelial venules (HEVs) and perhaps the follicular compartment [3,9]. Lymph nodes can undergo rapid and profound hypertrophy during an immune response and FRCs are deeply involved in matrix reorganization and the remodeling of lymph-node architecture [8]. In the lymph nodes of mice, the presence of cells with an intermediate FRC–SEC morphology suggests that these cell types are ontogenetically related [10].

DCs in the T-cell compartment

The lymph node harbors a composite population of DCs [11]. These are bone-marrow-derived cells that develop from immature antigen-capturing DCs that differentiate into potent priming cells for naïve T lymphocytes. In the mouse, at least six subsets of DCs have been identified on the basis of their morphology, distinct surface molecules and functional properties [12]. Three are blood-derived and enter the cortex through HEVs, the other three are tissue-derived and have access to the cortex through the subcapsular sinus via afferent lymph [13]. In humans, two primary subsets of DCs ($CD11c^+$ and $CD11c^-$ DCs) are distinguished based on their phenotype, morphology and function. $CD11c^+$ DCs are present as stellate cells whose delicate processes or 'veils' extend in many directions; these are called interdigitating DCs. $CD11c^-$ DCs show a morphology similar to typical plasma cells and are called plasmacytoid DCs [14]. Both DC subsets occupy the T-cell-dependent paracortical and interfollicular areas and form clusters with T lymphocytes. In particular, plasmacytoid DCs are clustered around the HEVs (Figure 2).

DCs in the B-cell compartment

A distinct type of DC, called the follicular DC (FDC), localizes in the B-cell-rich follicular areas. These cells occupy the outer layers of primary and secondary follicles – the light zone and the follicular mantle. FDCs constitute

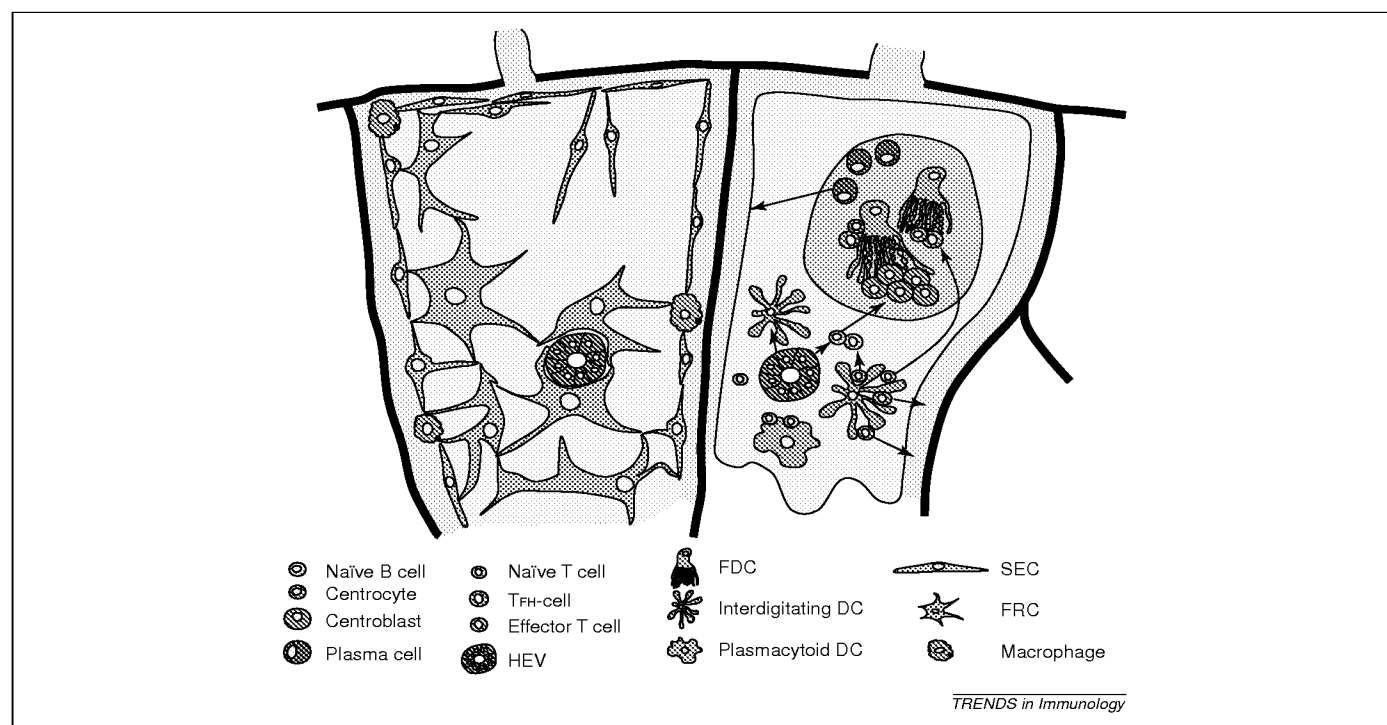


Figure 2. Microscopical architecture of a lymph node. The three-dimensional network of fibroblastic reticular cells (FRC) (left panel) provides the cellular basis for the conduit system, which connects the subcapsular and intermediate sinuses to high endothelial venules (HEV). The afferent lymph percolates through these lymphoid spaces lined by a single layer of sinus endothelial cells (SEC) and intermingled with macrophages. Lymph-borne molecules and locally produced 'homing' chemokines are transported to HEV, thus influencing lymphocyte trafficking. Naïve B and T cells (right panel) enter the lymph node through HEV in response to specific chemoattractants and migrate to distinct zones (arrows). B cells localize to lymph follicles, in response to CXCL13 cytokine secreted by follicular dendritic cells (FDC), and can initiate the germinal-center reaction, which leads to the formation of plasma cells with high-affinity for antigen binding. In the paracortex, T cells encounter dendritic cells (DC), which drive T-cell differentiation into either polarized cells ($CD4^+$ or $CD8^+$ effector T cells) or nonpolarized cells ($CD4^+$ memory or follicular helper T cells). Plasma cells and effector T cells eventually leave the lymph node through the intermediate and medullary sinuses (arrows).

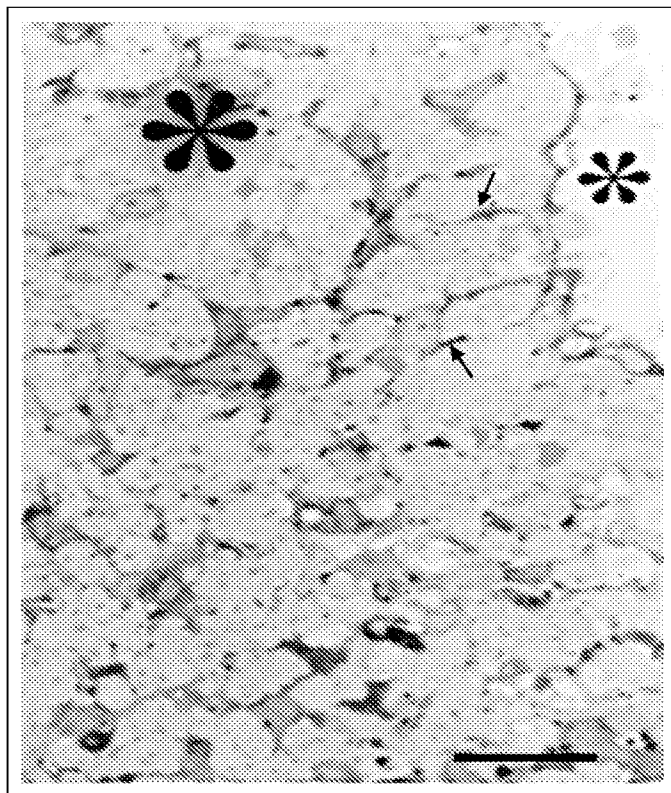


Figure 3. Microscopical view of a lymph node. Fibroblastic reticular cells (FRCs) are visualized in the mouse lymph node by a histochemical procedure employing osmium–zinc-iodide (E. Crivellato *et al.*, unpublished). FRC present an irregularly stellate shape with slender interconnected projections forming an extensive network in the paracortex and medulla. Two FRCs (arrows) connect the intermediate sinus (small asterisk) with the wall of a high endothelial venule (HEV) (large asterisk). In the paracortex, HEVs are almost completely wrapped by FRC. A single layer of sinus endothelial cells (SEC) lines the wall of the intermediate sinus. Similar to FRC, SECs are also reactive to osmium–zinc-iodide [10] suggesting a possible ontogenetic relationship between these cell types. Scale bar represents 20 μ m.

a three-dimensional sponge-like network formed by interdigitation of adjacent cells [15]. As shown by ultrastructural studies, these cells present filiform or beaded dendrites with characteristic ‘ball-of-yarn’ convolutions. FDCs play a pivotal role in promoting B-cell proliferation and differentiation in germinal centers [16]. In particular, FDCs specialize in stimulating potent B-cell recall responses, because they are very efficient in trapping and retaining antigen–antibody complexes, through CD21–CD35 and Fc γ RIIB receptors, for long periods and presenting them as iccosomes (immune-complex-coated bodies) to memory B cells [15,17]. Immune complexes are transported from the subcapsular sinus to FDCs either by subcapsular sinus macrophages [18] or, possibly, by cortical FRCs [3]. FDCs have also a remarkable structural function in organizing the primary lymphoid follicles, as shown by the absence of FDCs and follicles in TNF- α knockout mice [6]. FDCs are probably a heterogeneous cell population that can develop from hematopoietic precursors or by local differentiation from fibroblasts [16]. A recent study classifies six subtypes of FDCs in humans on the basis of their morphology,

phenotypic expression and ability to retain immune complexes [19].

Functional organization of the T-cell compartment

An adaptive immune response begins in the paracortex, when naïve T cells encounter resident DCs presumably taking up soluble antigens from the afferent lymph (Figure 2). By contrast, maintenance of the T-cell response depends on a later wave of immigrant DCs from the site of inflammation [20]. Naïve T cells constantly circulate through the blood and lymphoid tissues looking for their cognate antigens [21]. They express the CCR7 receptor and enter the paracortex through HEVs, in response to ‘homing’ chemokines expressed by HEVs, stromal cells and DCs, such as the secondary lymphoid organ chemokine (SLOC or CCL21) and the Epstein–Barr-virus-induced molecule-1 ligand chemokine (ELC or CCL19) [22,23]. DCs activate naïve CD4⁺ or CD8⁺ T cells by presenting antigen to them and providing costimulatory signals and cytokines [11]. Using real-time two-photon microscopy, it has been calculated that each DC can contact ~500 different T cells in an hour [24]. Specialized T cells emerge from this process. Whereas Th1 and Th2 CD4⁺ effector cells leave the lymph node through the efferent lymph and preferentially migrate to nonlymphoid tissues, nonpolarized CD4⁺ T cells express a lymphoid-tissue-homing phenotype [25,26]. They remain in the lymph nodes as either memory T cells or a T-cell subset specialized to provide help for B cells. This T-cell subset expresses CXC chemokine receptor 5 (CXCR5) and is highly responsive to the follicular homing chemokine B-lymphocyte chemoattractant (BLC or BCA-1 or CXCL13) secreted by FDCs [27,16]. Thus, these follicular B-helper CD4⁺ T cells (Tfh cells) enter the germinal center and promote terminal B-cell differentiation.

Functional organization of primary and secondary follicles

The anatomical organization of both primary and secondary lymphoid follicles is highly dependent upon FDCs, the expression of CXCL13 by which is essential for follicular clustering of B cells and attracting Tfh cells [16,28]. Similar to T cells, B cells enter the lymph node through HEVs within the T-cell zone, where B cells are subsequently guided to the follicles by specific chemoattractants (Figure 2). On antigen challenge, B cells migrate to the border of a primary follicle, where they meet antigen specific CD4⁺ T cells and initiate the formation of the germinal center. A fully mature secondary follicle presents a germinal center and a mantle zone. The germinal center is instrumental in the generation of memory B cells and plasma cells with high-affinity for antigen binding. It consists of two compartments – the dark and the light zones – occupied by phenotypically distinct B-cell populations referred to as centroblasts and centrocytes. Centroblasts are actively proliferating cells that undergo diversification of their B-cell receptor repertoire through somatic hypermutation of their IgV-region genes [29]. The resulting B cells move to the light zone and become centrocytes. Positive selection of centrocytes occurs through high-affinity binding to antigens trapped by FDCs. These

high-affinity B cells receive surviving signals both by FDCs and by Tfh cells located in the light zone, particularly at its outer edge, whereas low-affinity B cells do not gain access to antigens and die by apoptosis. Memory and antibody-forming B cells migrate to medullary cords, where the latter become plasma cells. They eventually leave the lymph node through the medullary sinus.

The functional anatomy of the spleen

General architecture of the spleen

The spleen is a highly vascular organ composed of the white and the red pulp. The white pulp is structurally similar to a lymph node and allows the generation of antigen-specific immune responses. In mammals, it consists of three compartments – periarteriolar lymphoid sheaths (PALS), lymph follicles and a marginal zone between the white and the red pulp (Figure 4). The framework of the white pulp is formed by a heterogeneous population of reticular fibroblasts ensheathing a delicate

network of type IV collagen fibers [30]. In the rat, these fibroblasts and fibers form the splenic conduit system, which strongly resembles the corresponding system of lymph nodes [31]. It transports blood-borne molecules or locally produced homing cytokines, such as CCL21 in the T-cell area and CXCL13 in the B-cell area, and provides a framework for directing lymphocyte migration and organization in the white pulp. PALS constitute a T-cell-dependent area in which T cells predominate, whereas the lymph follicles and marginal zone are B-cell-dependent areas. The lymph follicles are similar to those in the cortex of lymph nodes, with germinal centers and mantle zones. As in lymph nodes, interdigitating DCs and FDCs in the PALS and lymph follicles create distinct microenvironments and play a role in the homing mechanism of T and B cells, respectively (Figure 4) [32]. The marginal zone is the major route of entry to the white pulp for lymphocytes seeding into the spleen, blood-borne antigens and antigen presenting cells (APCs) [33]. It is specially equipped for the initiation of immune responses to T-cell-independent type II antigens [34].

Organization of the white pulp in rodents

The nomenclature for some parts of the white pulp was coined in rats and its structure has been thoroughly described in this species. Small branches of the splenic artery (i.e. the central arterioles) are concentrically surrounded by the PALS (Figure 4). At regular intervals, tightly packed accumulations of small strongly IgD⁺-recirculating B cells, the lymph follicles, are peripherally attached to the surface of the PALS. Their intense expression of surface IgD is a hallmark of recirculating B cells. PALS and lymph follicles are covered by the marginal zone, which delimits both regions from the red pulp. The marginal zone is composed of a special type of memory B cells expressing IgM but no or only minor amounts of IgD [35]. It also contains DCs, natural killer (NK) cells and two macrophage populations – metallophilic macrophages that accompany the marginal sinus and scattered macrophages that function as APCs [36]. The marginal zone is separated from the PALS and lymph follicles by the marginal sinus, a series of flattened leaky vascular structures into which part of the arterial bloodstream opens. Recirculating B and T cells enter the white pulp through this sinus and subsequently through the marginal zone.

Organization of the white pulp in humans

The normal microscopic anatomy of the human spleen is a controversial subject and the compartments of its white pulp are ill defined. The white pulp in human differs from that in rat in several ways (Figure 4) [37,38]. First, in adult humans, primary B-cell follicles occupy most of the white-pulp area. The PALS is not continuous and occurs around larger arteries. Central arterioles might run directly through follicles without being covered by T cells. Second, the marginal zone in human is primarily around the follicles and only a few IgM⁺IgD⁻ B cells (the typical marginal-zone phenotype) occur along the PALS. Third, there is no marginal sinus and hence the marginal zone is not separated from the primary and secondary follicles.

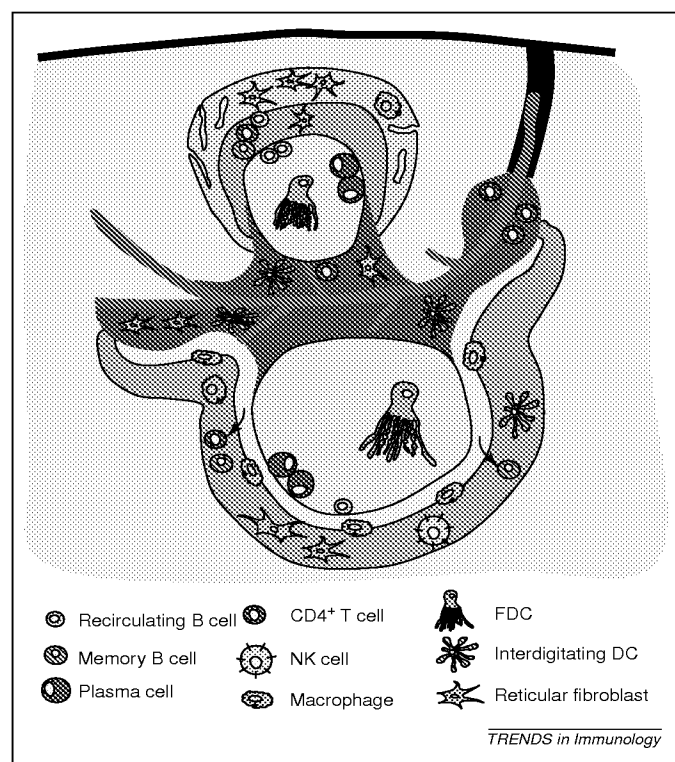


Figure 4. Structural and functional architecture of the white pulp in the spleen. The architecture of white pulp differs between humans and rodents in several aspects. In rodents (lower part of the figure), small branches of the splenic artery, also called central arterioles, are concentrically surrounded by a continuous wrap of lymphoid tissue, the periarteriolar lymphoid sheath (PALS) (violet) – a T-cell compartment. At regular intervals, B-cell-rich lymph follicles (pink–yellow) localize to the surface of PALS. The marginal zone (green), another B-cell compartment, covers the PALS and the lymph follicles and delimits both regions from the splenic red pulp (pink). The marginal zone is the major route of entry for B cells, T cells, antigen-presenting cells and blood-borne antigens (arrows) and is separated from PALS and lymph follicles by the marginal sinus. In humans (upper part of the figure), the central arterioles are covered by a discontinuous PALS and the marginal zone is mostly restricted to the area around the lymph follicles. Besides recirculating and memory B cells, the marginal zone harbors CD4⁺ T cells. The marginal sinus is absent and an additional region outside the marginal zone, the perifollicular zone (yellow), contains blood-filled spaces that belong to the open splenic circulation. Lymphocytes are believed to enter the white pulp through the perifollicular zone. Abbreviations: FDC, follicular dendritic cell; NK cell, natural killer cell.

Fourth, there is an additional region – the perifollicular zone – outside the marginal zone. It contains sheathed capillaries and blood-filled spaces without endothelial lining that belong to the open splenic circulation [39]. B cells of the marginal-zone phenotype form the broad inner part of the marginal zone but, in addition, there is a smaller outer marginal zone composed of B cells with recirculating phenotype ($\text{IgM}^+\text{IgD}^{2+}$). Shell-like accumulation of CD4^+ T cells is found at the border between these two zones. They are structurally related to fibroblasts of a special endothelial cell-like type that express α -actin (α -SMA) and myosin in smooth muscles, mucosal addressin adhesion molecule-1 (MAdCAM-1), vascular cell adhesion molecule-1 (VCAM-1) and vascular adhesion protein-1 (VAP-1), as well as thrombomodulin, cytokeratins 8 and 18 and Thy-1 [39]. These branched fibroblasts localize to the perifollicular zone, the outer marginal zone and the PALS and ensheath the bundles of reticular fibers. The human white-pulp follicles are thus embedded in a structure similar to an extension of the T-cell area into the marginal zone resulting in intricate interdigitation of the T- and B-cell areas.

Antigen entry and recirculating cells in the white pulp

One major difference in the anatomical organization of lymph nodes and the white pulp of the spleen is in the vascular architecture of these organs. Vascular constraints have, therefore, profound implications in the mode of antigen transport and cell recruitment. Indeed, lymph nodes drain antigens from the periphery through afferent lymphatics, whereas the spleen has no afferent lymphatic vessels and receives antigens directly from the blood. Moreover, the white pulp lacks HEVs and is equipped with a unique type of leaky microcirculation permitting lymphocytes to leave the blood under low shear stress. In rodents, the architectural structure of the marginal zone results in a strongly reduced blood flow that allows intimate contact between antigens and effector cells. Large molecules, such as bovine serum albumin, IgG and dextran, are unable to enter the white pulp, whereas smaller blood-borne molecules can directly permeate this compartment [31]. In humans, the perifollicular zone is part of the open splenic circulation and represents the entry compartment for recirculating lymphocytes into the white pulp. Recruitment of naïve CD4^+ T cells depends on endothelial-like fibroblasts in the perifollicular zone and outer marginal zone that secrete attracting chemokines and provide guiding routes for T cells into the PALS [39]. Similarly, recruitment of recirculating B cells into the outer marginal zone is thought to depend on distinct fibroblast subsets of the marginal zone. Definite proof of this is, however, lacking.

The functional anatomy of the thymus

The general thymic architecture

The thymus is an encapsulated gland that functions as a primary lymphoid organ to generate T cells bearing the $\alpha\beta$ form of the T-cell receptor (TCR). Intrathymic T-cell maturation proceeds from the fetal liver or bone-marrow-derived hemopoietic stem cells and includes thymocyte proliferation, lineage commitment and selection events. The gland undergoes remarkable age-related changes

consisting of size reduction and substitution with adipose tissue. When sectioned, the thymus displays a lobuled pattern with distinct cortical and medullary compartments. Fibroblasts and type-1 collagen-rich trabeculae branch off from the capsule and penetrate the cortex, where they are restricted to perivascular spaces. Structurally, it is made up of two major components – the stromal compartment and the hematolymphoid parenchyma (Figure 5). The former is the intrinsic stable component and primarily consists of a rich network of cytokeratin-positive cells collectively referred to as thymic epithelial cells (TECs). The latter encompasses the transient continuously flowing subsets of developing thymocytes, as well as a variety of APCs, including DCs, macrophages and small amounts of B cells. The term stroma is misleading because, in fact, this is the functional component of the thymus that regulates the programs that control thymocyte survival, lineage commitment and selection [40].

The structural organization of TECs

TECs are a heterogeneous population, showing profound phenotypic differences between cortex and medulla. Ultrastructurally, six TEC subsets have been described, each occupying well-defined intrathymic microanatomical domains. In the cortex, TECs are stellate, extended and highly reticulating elements. Some very large TECs (types 2 and 3) embrace numerous thymocytes (up to hundreds of thymocytes per cell) and create discrete micro-environmental niches in the outer cortex, called thymic nurse-cell (TNC) complexes [41] (Figure 6). Because TNCs express high levels of MHC class I and II molecules, and also contain acidic organelles necessary for antigen processing and presentation, they might be the primary sites of positive selection. In the medulla, TECs are less interconnected but more compact and heterogeneous.

The thymic epithelium provides a broad spectrum of both contact and paracrine signals for thymocyte proliferation, differentiation and selection. MHC-mediated TEC–thymocyte interactions play a central role in T-cell selection. TECs also secrete cytokines (IL-1, -2, -3, -6 and GM-CSF), chemokines [42] and peptides (thymic hormones and neuropeptides) [43]. Moreover, the thymic epithelium is the main source of the components of extracellular matrix (ECM), such as laminin, fibronectin and type IV collagen, which are thought to provide pre-formed routes for thymocyte migration inside the organ [42]. ECM proteins, such as laminin isoforms, are also implicated in thymocyte differentiation. In the mouse thymus, for instance, progression of cells from double negative (DN: $\text{CD4}^-\text{CD8}^-$) to double positive (DP: $\text{CD4}^+\text{CD8}^+$) is believed to require laminin-mediated interactions [44,45]. In addition, ECM-mediated epithelial–thymocyte interactions play a role in the traffic of thymocytes within TNC complexes, affecting both the entrance and exit of T cells in this particular niche [42,45].

Thymocyte proliferation and maturation: the process of thymocyte selection

The interstitial labyrinthine spaces lined by the epithelial meshwork harbor the moving population of proliferating

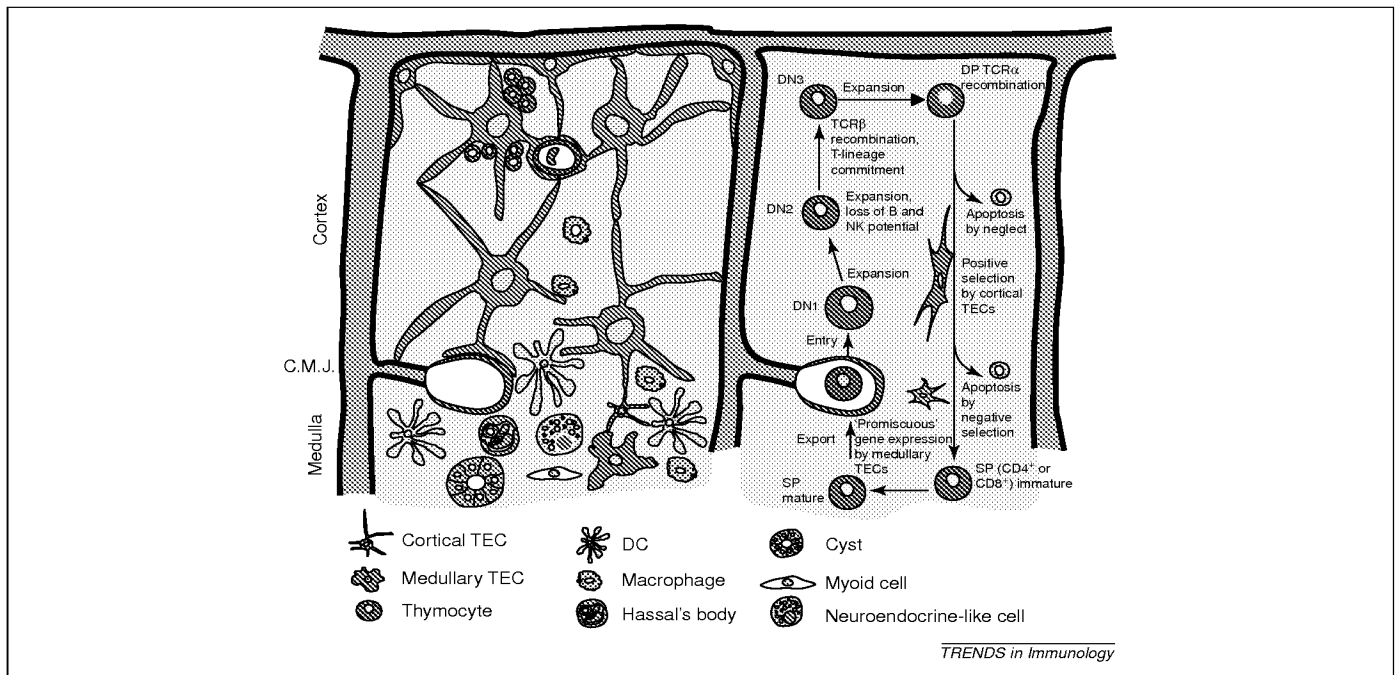


Figure 5. Structural and functional architecture of the thymus. Resident thymic epithelial cells (TEC) (left panel) form the infrastructure of the gland. They provide developing thymocytes with both differentiation signals and guiding routes. The medulla harbors dendritic cells (DC), macrophages and ectopic structures (Hassal's bodies, myoid cells, epithelial cysts and neuroendocrine-like cells), which have recently been implicated in the process of central tolerance. Immigrant hemopoietic cells (right panel) enter the thymus through large venules at the corticomedullary junction and undergo a program of proliferation, lineage commitment and MHC-restricted selection of T-cell receptor (TCR) $\alpha\beta$ (positive selection) under the influence of cortical TEC. Although negative selection caused by unsuccessful MHC–TCR interactions occurs in the cortex, it is the medulla that is the main site for deletion of autoreactive thymocytes. Selected single-positive $CD4^+$ or $CD8^+$ thymocytes undergo final maturation in the medulla and are then exported to the peripheral T-cell pool as fully functional self-tolerant T cells. Abbreviations: DN, double-negative thymocyte; DP, double-positive thymocyte; SP, single-positive thymocytes.

and differentiating thymocytes. Lymphopoiesis and the functional maturation of thymocytes are associated with cell migration across distinct intrathymic subregions (Figure 5). Uncommitted hemopoietic progenitors leave the bloodstream and enter the organ in a narrow region at the perimedullary cortex (zone 1, the inner 15–20% of the cortex) [46]. Cell extravasation occurs at large post-capillary venules located at the corticomedullary junction. Thymic-homing precursors proliferate and move outward across the cortex to the subcapsular region (zone 4). They pass through spatially and functionally defined cortical domains (zones 2 and 3) and progressively acquire commitment to the T lineage [47]. This process requires active signaling, such as Notch-1–Jagged interactions, mediated by cortical TECs. Thymocyte accumulation in the subcapsular region is accompanied by transition from DN3 to DP. In turn, early DP cells begin their journey back to the medulla. Moving inward across the cortex, they undergo either positive selection (rescuing of MHC-restricted thymocytes from programmed cell death) or negative selection (apoptosis by neglect) by TCR scanning of self-peptide–MHC complexes displayed on cortical TECs. Only a minority (~4%) of DP cells are positively selected to generate mature single-positive (SP) cells. Once the SP phenotype is acquired, thymocytes enter the medulla where they remain for a few days before being released into the peripheral lymphoid pool.

The structural organization of thymic medulla: the process of central tolerance

The medulla has a distinct structural pattern. It harbors a highly heterogeneous population of TECs (1–5% of medullary TECs) expressing a mosaic of 'ectopic' tissue-specific molecules, such as parathyroid hormone, thyroglobulin, insulin and C-reactive protein ('promiscuous' gene expression) [48,49,50] (Figure 5). Remarkably, the thymic medulla contains stable groups of cells displaying the organization, morphology and functional activity of other epithelial tissues, namely Hassal's bodies (squamous epithelial cells that resemble epidermal epithelium), cystic organoid structures with the morphological and phenotypic features of respiratory epithelium, neuroendocrine cells, myoid cells and solitary thyroid follicles [50,51] (Figure 5). This organization is thought to maintain the spectrum of epithelial 'self' in the thymus (immunological homunculus) with profound implications for the mechanisms of central tolerance [52,53]. In addition, the medulla is densely packed with non-epithelial APCs, such as macrophages and DCs, of both lymphoid and myeloid origin. DCs, in particular, are considered to have major functions in the process of deletion of T-cell autoreactive clones (hemopoietic self) [53,54]. Surprisingly, the medulla harbors a small population of B cells, which are also believed to contribute to tolerance induction in a poorly defined mode [55].

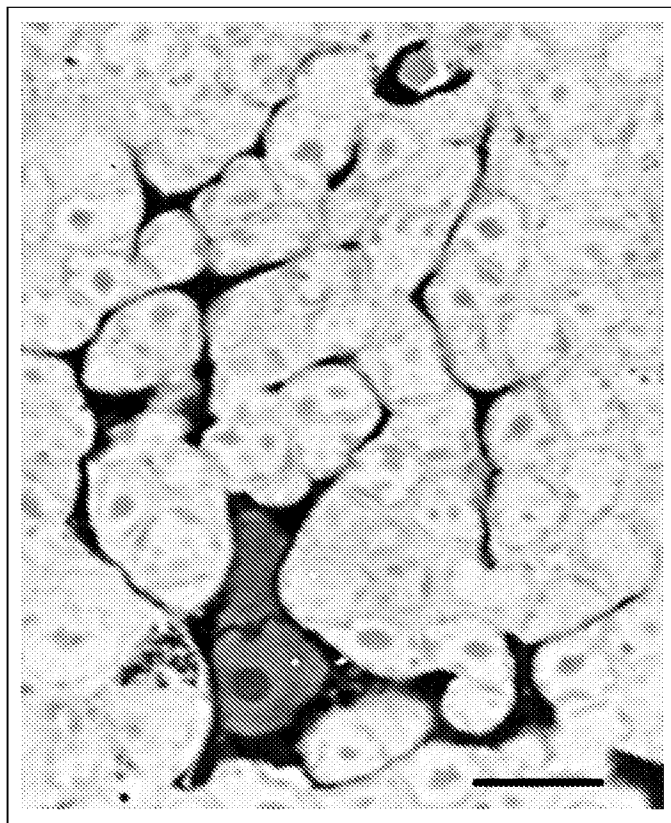


Figure 6. Microscopical view of a thymic cortex. A thymic nurse-cell (TNC) is visualized in the mouse thymus by a histochemical procedure employing osmium–zinc–iodide (E. Crivellato *et al.*, unpublished). TNCs are large epithelial cells located in the outer cortex. With their extended cytoplasmic projections, they embrace hundreds of thymocytes and create discrete microenvironmental niches, which provide facility for extensive contact between the plasma membranes of TNCs and thymocytes. TNC–thymocyte complexes might be the primary sites of positive selection of thymocyte. Indeed, TNCs present high levels of MHC class I and II molecules, which interact with the T-cell receptor expressed on the surface of cortical thymocytes. Scale bar represents 10 μ m.

The crosstalk between developing thymocytes and TECs is crucial for the induction of both a typical thymic architecture and negative selection. In the mouse, the product of the autoimmune regulator (AIRE) gene is a candidate molecule for such a pivotal interaction and its expression correlates with a normal stromal organization [56,57]. AIRE expression is mainly restricted to the subpopulation of Ag29⁺ TECs located within the medulla and the corticomedullary junction. In adult mice, Ag29⁺ TECs form a network of scattered medullary stromal cells with abundant and reticulated cell processes. Mutations in the AIRE gene are responsible for an autoimmune syndrome called APECED (autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy) and are characterized by loss of selftolerance in multiple organs. The first AIRE-specific transcripts are detected at embryonic day 14 (E14), in parallel with the appearance of DN2 thymocytes. On E16, a network of Ag29⁺ medullary stromal cells is established, in parallel with the emergence of more mature thymocytes that express *Rel B*, a transcription factor of the NF- κ B family. In *Rel-B*-deficient mice, activated TECs cannot persist, and these cells are

disorganized [58]. Approximately 5% of peripheral CD4⁺ T cells show autoreactivity, suggesting that positively selected thymocytes are not totally deleted from autoreactive cells. If optimal organization of thymic medullary stroma is required for the induction of tolerance, its disruption indicates autoimmune disease.

Concluding remarks and perspectives

The development of fully competent T cells, as well as the initiation and regulation of adaptive immune responses, is greatly facilitated by anatomical strategies. Understanding the structural organization of a lymphoid organ has profound implications for comprehending many important aspects of immune function. It enables the scientist to place hypotheses and theoretical models, drawn from *in vitro* experiments in cell and molecular immunology, into a real anatomical context. In the rapid development of immunological concepts, exploitation of old and new microscopic technologies, such as *in situ* video-microscopic analysis with time-lapse two-photon microscopy [59,60], will certainly throw light on some basic aspects of immunobiology, such as tissue- and microenvironment-selective homing of lymphocytes and accessory cell subsets, specificity and duration of cell-to-cell contacts, cell movement and interaction with the stromal matrix and, pathways and distribution of labeled antigens or tracer molecules.

Acknowledgements

This work was supported in part by grants from the Italian Ministry for Education, University and Research, MIUR (Interuniversity Funds for Basic Research - FIRB- and local funds), Rome, Italy.

References

- Gretz, J.E. *et al.* (2000) Lymph-borne chemokines and other low molecular weight molecules reach high endothelial venules via specialized conduits while a functional barrier limits access to the lymphocyte microenvironments in lymph node cortex. *J. Exp. Med.* 192, 1425–1439
- Van Vliet, E. *et al.* (1986) Reticular fibroblasts in peripheral lymphoid organs identified by a monoclonal antibody. *J. Histochem. Cytochem.* 34, 883–890
- Crivellato, E. and Mallardi, F. (1997) Stromal cell organisation in the mouse lymph node. A light and electron microscopic investigation using the zinc iodide-osmium technique. *J. Anat.* 190, 85–92
- Gretz, J.E. *et al.* (1997) Cords, channels, corridors and conduits: critical architectural elements facilitating cell interactions in the lymph node cortex. *Immunol. Rev.* 156, 11–24
- Delgado, E.V. *et al.* (1998) Mature dendritic cells respond to SDF-1, but not to several β -chemokines. *Immunobiology* 198, 490–500
- Fu, Y.X. and Chaplin, D.D. (1999) Development and maturation of secondary lymphoid tissues. *Annu. Rev. Immunol.* 17, 399–433
- Ngo, V.N. *et al.* (1999) Lymphotoxin $\alpha\beta$ and tumor necrosis factor are required for stromal cell expression of homing chemokines in B and T cell areas of the spleen. *J. Exp. Med.* 189, 403–412
- Di Sciuillo, G. *et al.* (1998) L1 antibodies block lymph node fibroblastic reticular matrix remodeling *in vivo*. *J. Exp. Med.* 187, 1953–1963
- Baekkevold, E.S. *et al.* (2001) The CCR7 ligand ELC (CCL19) is transcytosed in high endothelial venules and mediates T cell recruitment. *J. Exp. Med.* 193, 1105–1112
- Crivellato, E. and Mallardi, F. (1998) The sinus endothelial cell architecture in the mouse lymph node. Structural peculiarities and close correlation with the fibroblastic reticular cells. *J. Submicrosc. Cytol. Pathol.* 30, 495–502
- Banchereau, J. and Steinman, R.M. (1998) Dendritic cells and the control of immunity. *Nature* 382, 245–252

- 12 Ardavin, C. *et al.* (2001) Origin and differentiation of dendritic cells. *Trends Immunol.* 22, 691–700
- 13 Itano, A.A. and Jenkins, M.J. (2003) Antigen presentation to naive CD4 T cells in the lymph node. *Nat. Immunol.* 4, 733–739
- 14 Cella, M. *et al.* (2000) Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent Th1 polarization. *Nat. Immunol.* 1, 305–310
- 15 Tew, J.G. *et al.* (1997) Follicular dendritic cells and presentation of antigen and costimulatory signals to B cells. *Immunol. Rev.* 156, 39–52
- 16 Cyster, J.G. *et al.* (2000) Follicular stromal cells and lymphocyte homing to follicles. *Immunol. Rev.* 176, 181–193
- 17 Barrington, R.A. *et al.* (2002) B lymphocyte memory: role of stromal cell complement and FcγRIIB receptors. *J. Exp. Med.* 196, 1189–1199
- 18 Martinez-Pomares, I. *et al.* (1996) Fc chimeric protein containing the cysteine-rich domain of the murine mannose receptor binds to macrophages from splenic marginal zone and lymph node subcapsular sinus and to germinal centers. *J. Exp. Med.* 184, 1927–1937
- 19 Bofil, M. *et al.* (2000) Follicular dendritic cells share a membrane-bound protein with fibroblasts. *J. Pathol.* 191, 217–226
- 20 Itano, A.A. *et al.* (2003) Distinct dendritic cell populations sequentially present antigen to CD4 T cells and stimulate different aspects of cell-mediated immunity. *Immunity* 19, 47–57
- 21 Butcher, E.C. and Picker, L.J. (1996) Lymphocyte homing and homeostasis. *Science* 272, 60–66
- 22 Gunn, M.D. *et al.* (1998) A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. *Proc. Natl. Acad. Sci. U. S. A.* 95, 258–263
- 23 Ngo, V.N. *et al.* (1998) Epstein–Barr virus-induced molecule 1 ligand chemokine is expressed by dendritic cells in lymphoid tissues and strongly attracts naive T cells and activated B cells. *J. Exp. Med.* 188, 181–191
- 24 Bousso, P. and Robey, E. (2003) Dynamics of CD8⁺ T cell priming by dendritic cells in intact lymph nodes. *Nat. Immunol.* 4, 579–585
- 25 Masopust, D. *et al.* (2001) Preferential localization of effector memory cells in nonlymphoid tissue. *Science* 291, 2413–2417
- 26 Iezzi, G. *et al.* (2001) Migration and function of antigen-primed nonpolarized T lymphocytes. *J. Exp. Med.* 193, 987–993
- 27 Breitfeld, D. *et al.* (2000) Follicular B-helper T cells express CXC chemokine receptor 5, localize to B-cell follicles and support immunoglobulin production. *J. Exp. Med.* 192, 1545–1552
- 28 Moser, B. *et al.* (2002) CXCR5⁺ T cells: follicular homing takes center stage in T-helper-cell responses. *Trends Immunol.* 23, 250–254
- 29 Van Eijk, M. *et al.* (2001) Death-receptor contribution to the germinal-center reaction. *Trends Immunol.* 22, 677–681
- 30 Satoh, T. *et al.* (1997) Immunohistochemical and structural characteristics of the reticular framework of the white pulp and marginal zone in the human spleen. *Anat. Rec.* 249, 486–494
- 31 Nolte, M.A. *et al.* (2003) A conduit system distributes chemokines and small blood-borne molecules through the splenic white pulp. *J. Exp. Med.* 198, 505–512
- 32 Buckley, J.J. *et al.* (1987) Human spleen contains phenotypic subsets of macrophages and dendritic cells that occupy discrete microanatomic locations. *Am. J. Pathol.* 128, 505–520
- 33 Martin, F. *et al.* (2001) Marginal zone and B1 B cells unite in the early response against T-independent blood-borne particulate antigens. *Immunity* 14, 617–629
- 34 Spencer, J. *et al.* (1998) Human marginal-zone B cells. *Immunol. Today* 19, 421–426
- 35 Kraal, G. (1992) Cells in the marginal zone of the spleen. *Int. Rev. Cytol.* 132, 31–73
- 36 Dijkstra, C.D. *et al.* (1985) Marginal zone macrophages identified by a monoclonal antibody: characterization of immuno- and enzyme-histochemical properties and functional capacities. *Immunology* 55, 23–30
- 37 Steiniger, B. *et al.* (1997) The species-specific structure of microanatomical compartments in the human spleen: strongly sialoadhesin-positive macrophages occur in the perifollicular zone, but not in the marginal zone. *Immunology* 92, 307–316
- 38 Steiniger, B. *et al.* (2003) The three-dimensional structure of human splenic white pulp compartments. *J. Histochem. Cytochem.* 51, 655–663
- 39 Steiniger, B. *et al.* (2001) The perifollicular and marginal zones of the human splenic white pulp. Do fibroblasts guide lymphocyte immigration? *Am. J. Pathol.* 159, 501–512
- 40 Anderson, G. and Jenkinson, E.J. (2001) Lymphostromal interactions in thymic development and function. *Nat. Rev. Immunol.* 1, 31–40
- 41 Brelinska, R. and Warchol, J.B. (1997) Thymic nurse cells: their functional ultrastructure. *Microsc. Res. Tech.* 38, 250–266
- 42 Savino, W. *et al.* (2002) Intrathymic T-cell migration: a combinatorial interplay of extracellular matrix and chemokines? *Trends Immunol.* 23, 305–313
- 43 Mentlein, R. and Kendall, M.D. (2000) The brain and thymus have much in common: a functional analysis of their microenvironments. *Immunol. Today* 21, 133–140
- 44 Magner, W.J. *et al.* (2000) Aberrant development of thymocytes in mice lacking laminin-2. *Dev. Immunol.* 7, 179–193
- 45 Kim, M.G. *et al.* (2000) Epithelial-cell-specific laminin-5 is required for survival of early thymocytes. *J. Immunol.* 165, 192–201
- 46 Lind, E.F. *et al.* (2001) Mapping precursor movement through the postnatal thymus reveals specific microenvironments supporting defined stages of early lymphoid development. *J. Exp. Med.* 194, 127–134
- 47 Petrie, H.T. (2002) Role of thymic organ structure and stromal composition in steady-state post-natal T-cell production. *Immunol. Rev.* 189, 8–19
- 48 Farr, A.G. and Rudensky, A. (1998) Medullary thymic epithelium: a mosaic of epithelial “self”? *J. Exp. Med.* 188, 1–4
- 49 Derbinski, J. *et al.* (2001) Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. *Nat. Immunol.* 2, 1032–1039
- 50 Farr, A.G. *et al.* (2002) Organization of thymic medullary epithelial heterogeneity: implications for mechanisms of epithelial differentiation. *Immunol. Rev.* 189, 20–27
- 51 Botham, C.A. *et al.* (2001) Immuno-characterization of neuroendocrine cells of the rat thymus gland *in vitro* and *in vivo*. *Cell Tissue Res.* 303, 381–389
- 52 Klein, L. and Kyewski, B. (2000) Self-antigen presentation by thymic stromal cells: a subtle division of labor. *Curr. Opin. Immunol.* 12, 179–186
- 53 Kyewski, B. *et al.* (2002) Promiscuous gene expression and central T-cell tolerance: more than meets the eye. *Trends Immunol.* 23, 364–371
- 54 Sprent, J. and Kishimoto, H. (2002) The thymus and negative selection. *Immunol. Rev.* 185, 126–135
- 55 Kendall, M.D. and Clarke, A.G. (2000) The thymus in the mouse changes its activity during pregnancy: a study of the microenvironment. *J. Anat.* 197, 393–411
- 56 Zuklys, S. *et al.* (2000) Normal thymic architecture and negative selection are associated with Aire expression, the gene defective in autoimmune–polyendocrinopathy–candidiasis–ectodermal dysplasia (APECED). *J. Immunol.* 165, 1976–1983
- 57 Ramsey, C. *et al.* (2002) Systematic mutagenesis of the functional domains of AIRE reveals their role in intracellular targeting. *Hum. Mol. Genet.* 11, 3299–3308
- 58 Naquet, P. *et al.* (1999) Development, organization and function of the thymic medulla in normal, immunodeficient or autoimmune mice. *Semin. Immunol.* 11, 47–55
- 59 Robey, E.A. and Bousso, P. (2003) Visualizing thymocyte motility using 2-photon microscopy. *Immunol. Rev.* 195, 51–57
- 60 Wei, S.H. *et al.* (2003) A stochastic view of lymphocyte motility and trafficking within the lymph node. *Immunol. Rev.* 195, 136–159